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Research Article

Synthesis of tritium labelled 3(*R*)-HETE and 3(*R*),18(*R*/*S*)-DiHETE through a common synthetic route

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Summary

An efficient procedure for the synthesis of 3-hydroxyoxylipins labelled with tritium on all double bond positions is reported. The synthetic scheme involves a joint route for the formation of tetraacetylenic precursors followed by stereoselective reduction of the triple bonds either with hydrogen or tritium. The final tritiated products were obtained with specific activities ranging from 1.65 to 1.80 Ci/mmol. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: tritium labelled fatty acids; 3(*R*)-hydroxy-oxylipins; 3(*R*)-HETE; 3(*R*),18(*R*/*S*)-DiHETE

Introduction

3(*R*)-Hydroxy fatty acid derivatives of AA, a group of biologically active commonly named 3(*R*)-hydroxy-oxylipins, were identified in some fungal species (*Dipodascopsis uninucleata and Mucor genevensis*)[1–3] when exogenous

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AA was utilized as a substrate. 3(*R*)-hydroxy-oxylipins have been shown to be potent microbial growth factors[4,5] and to play a crucial role in the morphogenesis of *Candida albicans*.[4] Moreover, 3-HETE was found to be a strong proinflammatory lipid mediator, which modulates several human neutrophil functions, such as chemotaxis and phagocytosis. Recently, a novel AA metabolite – 3,18-DiHETE – has been identified in *Candida albicans*.[4] Moreover, its formation was strongly suppresed by salicylic acid and its acetylated derivative. Despite progress in the research of 3(*R*)-hydroxy-oxylipins, a number of open issues on their role in intracellular signal transduction pathways needs to be clarified. The site of the formation of 3,18-DiHETE still remains to be established, and whether its formation affects the pattern of endogenous 3(*R*)-hydroxy-oxylipins, and if the various oxylipins reveal different biological activities. The utilization of radiolabelled analogues will facilitate the detection of small changes in metabolic products. Hence, efficient synthetic methods for their preparation are required to understand the extensive biological role of 3(*R*)-hydroxy-oxylipins in mammalian cells. In the present study we describe simple and effective method for the synthesis of 3(*R*)-HETE, and 3(*R*),18(*R*/*S*)-DiHETE, selectively labelled with tritium on all double bond positions.

Experimental

^{1}H and ^{13}C NMR spectra for compounds **5**–**7** were recorded either on a Bruker MSL 200 or Bruker MSL 300 spectrometers in $CDCl_3$ as solvent. Chemical shifts are referenced to tetramethylsilane as an internal standard for ^{1}H NMR or to the deuterium lock signal of $CDCl_3$ ($\delta^{13}C = 77.19$ ppm). HPLC analysis was carried out on a Shimadzu LC-10Avp liquid chromatograph connected to a SPD-10Advp UV detector. RP-HPLC analysis was performed on a Nucleosil C18-column; 250 × 4 mm, 5 μm particle size (Machery-Nagel, Düren, Germany) with different solvent systems: $MeOH/H_2O$ (95:5, by vol.) and a flow rate of 1 ml/min were used for analysis of compounds **6**, **8** and $MeOH/H_2O/Ac$ (85:15:0.1, by vol.) for acid **2** and $MeOH/H_2O/Ac$ (75:25:0.1, by vol.) for dihydroxy acids **7** and **9**. Preparative HPLC was carried out on a Lichrospher 100 RP18 column; 250 × 22.5 mm, 10 μm particle size (Knauer, Berlin, Germany) with $MeOH/H_2O/AcOH$ (95:5, by vol.) and a flow rate of 10 ml/min. For ESI MS analysis a Finnigan MAT-900XL mass-spectrometer was used. Column chromatography was carried out on Silica Gel 60 (Merck, Darmstadt, Germany particle size ranging from 70–230 mesh). For thin-layer chromatography we employed preformed Silica Gel 60 F_{254} sheets (Merck, Darmstadt, Germany). All solvents and reagents used were of extra pure grade and purchased from Merck, Aldrich or Across (Germany). Prior to use all glassware and syringes were dried at 140°C overnight and all reactions were carried out under an atmosphere of dry argon.

*[5,6,8,9,11,12,14,15-^{3}H]-(3R,5Z,8Z,11Z,14Z)-3-Hydroxyeicosa-5,8,11,14-tetraenoic acid (**2**)*

A solution of **1** (20 mg, 0.06 mmol) in benzene (1 ml), Lindlar's catalyst (35 mg) and quinoline (0.03 ml) were placed in the reaction ampoule and the mixture was frozen with liquid nitrogen. After evacuation the ampoule was filled with a hydrogen–tritium mixture, containing 1% of tritium, up to a pressure of 400 hPa. The reaction mixture was stirred for 1 h at room temperature and then frozen again. Unreacted tritium gas was removed by applying vacuum. The catalyst was removed by filtration over silica gel and washed with benzene (3 × 1 ml). The combined filtrates were concentrated under reduced pressure and the residue was evaporated twice with methanol (2 × 2 ml) to remove labile tritium. The crude product was purified by preparative HPLC followed by saponification using LiOH (7 mg, 0.3 mmol) in water–methanol solution. After the reaction was completed, methanol was removed by evaporation under reduced pressure and the residue was carefully acidified to pH 4.0 using HCl (1 M). The lipophilic products were extracted with Et_2O (3 × 5 ml), combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by filtration over silica gel (hexane/Et_2O, 1:3) to yield 64% of acid **2** with a specific radioactivity of 1.65–1.75 Ci/mmol. The radiochemical purity of [^{3}H-8]-**2** was found to be 95%.

*Methyl (3R,18R/S)-3-Hydroxy-18-(benzoyloxy)eicosa-5,8,11,14-tetraynoate (**5**)*

In an argon filled previously dried, round-bottomed flask equipped with magnetic stirrer anhydrous K_2CO_3 (108 mg, 0.78 mmol), NaI (156 mg, 1.04 mmol) and CuI (198 mg, 1.04 mmol) were introduced followed by DMF (7 ml). Methyl 3(*R*)-hydroxy-5-hexynoate (**4**) (74 mg, 0.52 mmol) was added at once to the suspension followed by rac-3-(benzoyloxy)-14-bromotetradeca-6,9,12-triyne (**3**) (200 mg, 0.52 mmol) each in DMF (2 ml). The resulting suspension was vigorously stirred overnight at room temperature. The mixture was then quenched with saturated aqueous solution of NH_4Cl (200 ml) and the lipophilic products were extracted with Et_2O (3 × 80 ml). The combined organic extracts were washed with a saturated aqueous solution of NaCl (2 × 100 ml) and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography (hexane/Et_2O, 1:2) under argon to give pure **5** as a yellow oil. Yield of **5**: 187 mg (80%). TLC: $R_f = 0.29$ (hexane/Et_2O, 1:3). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.01 (m, 2H, *o*-Bz), 7.52 (m, 1H, *p*-Bz), 7.42 (m, 2H, *m*-Bz), 5.10 (m, 1H, 18-CH), 4.12 (m, 1H, 3-CH), 3.65 (s, 3H, OCH_3), 3.10 (m, 4H) and 3.02 (m, 2H, 7-,10- and 13-CH_2), 2.65 (dd, 1H, $J_1 = 3.8$ Hz, $J_2 = 16.5$ Hz) and 2.50 (dd, 1H, $J_1 = 8.8$ Hz, $J_2 = 16.5$ Hz, 2-CH_2), 2.39 (m, 2H, 4-CH_2), 2.22 (m, 2H, 16-CH_2), 1.85 (m, 2H, 17-CH_2), 1.67

(m, 2H, 19-CH_2), 0.92 (t, 3H, $J = 7.6$ Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.67, 166.39, 132.83, 131.07, 129.74 (2C), 128.46 (2C), 82.08, 80.02, 76.38, 75.42 (2C), 75.31, 74.76, 74.50, 74.32, 67.01, 51.75, 40.40, 33.04, 27.13, 27.06, 15.41, 9.93 (3C), 9.56.

*Methyl (3R,18R/S,5Z,8Z,11Z,14Z)-3-Hydroxy-18-(benzoyloxy)eicosa-5,8,11,14-tetraenoate (**6**)*

A suspension of Lindlar's catalyst (374 mg) in dry benzene (10 ml) was saturated with H_2 at room temperature and then cooled to 10°C. The ester **5** (187 mg, 0.42 mmol) in benzene (20 ml) and quinoline (0.37 ml) were added to the catalyst suspension under a stream of argon. The argon was subsequently exchanged with H_2 and the reaction mixture was stirred for 1 h at 10°C. H_2 uptake was measured with a gas burette. After the H_2 absorption was complete, the mixture was filtered and the filtrate was washed with HCl (2 M, 2 × 50 ml). Evaporation of the solvent yielded a crude residue, which was purified by preparative RP-HPLC (MeOH/H_2O, 95:5) to afford 243 mg (64%) of pure **6**. TLC: $R_f = 0.39$ (Et_2O/hexane, 3:1). RP-HPLC: $R_t = 4.25$ min (MeOH/H_2O/AcOH, 95:5:0.1). ^1H NMR (200 MHz, $CDCl_3$): δ 8.01 (m, 2H, *o*-Bz), 7.52 (m, 1H, *o*-Bz), 7.42 (m, 2H, *o*-Bz), 5.21–5.41 (m, 8H, CH = CH), 5.10 (m, 1H, 18-CH), 4.05 (m, 1H, 3-CH), 3.65 (s, 3H, OCH_3), 2.70 (m, 6H, 7-, 10-, 13-CH_2), 2.42 (m, 2H, 2-CH_2), 2.32 (m, 2H, 4-CH_2), 2.16 (m, 2H, 16-CH_2), 1.60–1.80 (m, 6H, 17- and 19-CH_2), 0.90 (t, 3H, $J = 6.8$ Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.04, 166.46, 132.76, 131.29 (2C), 129.71 (2C), 129.62, 129.45, 128.64, 128.57, 128.46 (2C), 128.24, 128.06, 125.11, 75.98, 68.18, 51.68, 40.75, 34.77, 33.89, 27.31, 26.03, 25.84, 23.53, 9.63. ESI MS *m*/*z*: 477 $[M + Na]^+$.

*(3R,18R/S,5Z,8Z,11Z,14Z)-3,18-Dihydroxyeicosa-5,8,11,14-tetraenoic acid (**7**)*

An aqueous solution of NaOH (0.33 M, 10 ml) was added under argon to a solution of ester **6** (102 mg, 0.22 mmol) in MeOH (15 ml). The mixture was stirred for 40 h at room temperature, MeOH was removed by evaporation, and the pH was adjusted carefully to 5.0 using HCl (1 M). Lipophilic products were then extracted with Et_2O (3 × 40 ml), the ethereal extracts dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The products were purified by flash chromatography (Et_2O/hexane, 5:1) to yield 59 mg (79%) of **7**. RP-HPLC: $R_t = 8.15$ min MeOH/H_2O/AcOH (75:25:0.1, by vol.). TLC: $R_f = 0.21$ ($CHCl_3$/MeOH, 9:1). ^1H NMR (300 MHz, $CDCl_3$): δ 5.55 (m, 1H) and 5.30–5.40 (m, 7H, CH = CH), 4.05 (m, 1H, 3-CH), 3.55 (m, 1H, 18-CH), 2.85 (m, 6H, 7-, 10-, 13-CH_2), 2.56 (dd, 1H, $J_1 = 3.8$, $J_2 = 16.5$) and 2.45 (dd, 1H, $J_1 = 7.9$, $J_2 = 16.5$, 2-CH_2), 2.31 (m, 2H, 4-CH_2), 2.14 (m, 2H, 16-CH_2), 1.41–1.59 (m, 4H, 17- and 19-CH_2), 0.87 (t, 3H, $J = 6.8$ Hz, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 177.20, 131.39, 129.62, 128.38 (3C), 127.93, 127.68, 124.55, 73.30, 67.88, 40.14, 36.30, 34.39, 30.15, 25.87, 25.72, 25.67, 23.55, 9.85.

*[5,6,8,9,11,12,14,15-^{3}H]-(3R,18R/S,5Z,8Z,11Z,14Z)-3-Hydroxy-18-(benzoyloxy)eicosa-5,8,11,14-tetraenoic acid (**8**)*

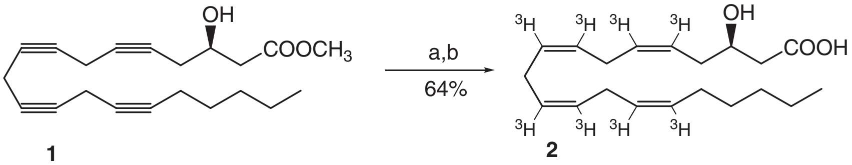
Compound **8** was prepared from methyl (3*R*,18*R*/*S*)-3-hydroxy-18-(benzoyloxy)eicosa-5,8,11,14-tetraynoate (**5**) (20 mg, 0.045 mmol) using Lindlar's catalyst (40 mg) and quinoline (0.4 ml) in a similar manner as described for the tritiation of tetraacetylene **1**. The crude product was purified by RP-HPLC (MeOH/H_2O, 95:5) to afford 56% of [$^3H_8$]-**8**, specific radioactivity 1.70–1.80 Ci/mmol. Compound **8** was found to be identical with its non-labeled analogue **6** as revealed by analytical RP-HPLC using a solvent system (MeOH/H_2O/AcOH, 95:5:0.1).

*[5,6,8,9,11,12,14,15-^{3}H]-(3R,18R/S,5Z,8Z,11Z,14Z)-3,18-Dihydroxyeicosa-5,8,11,14-tetraenoic acid (**9**)*

An aqueous solution (3 ml) of NaOH (15 mg, 0.378 mmol) was added to a solution of **8** (43 mCi, 0.025 mmol) in methanol (12 ml) under an argon atmosphere. The resulting mixture was stirred for 30 h at room temperature. After the reaction was complete, methanol was removed by evaporation. The residue was acidified using HCl (1 M), the products were extracted with Et_2O (4 × 2 ml) and the combined organic extracts were concentrated under reduced pressure. The crude residue was filtered over silica gel ($CHCl_3$/MeOH, 9:1) to give 86% of **9** with a radiochemical purity of 97%; the specific activity of 1.70–1.80 Ci/mmol remained unchanged.

Results and discussion

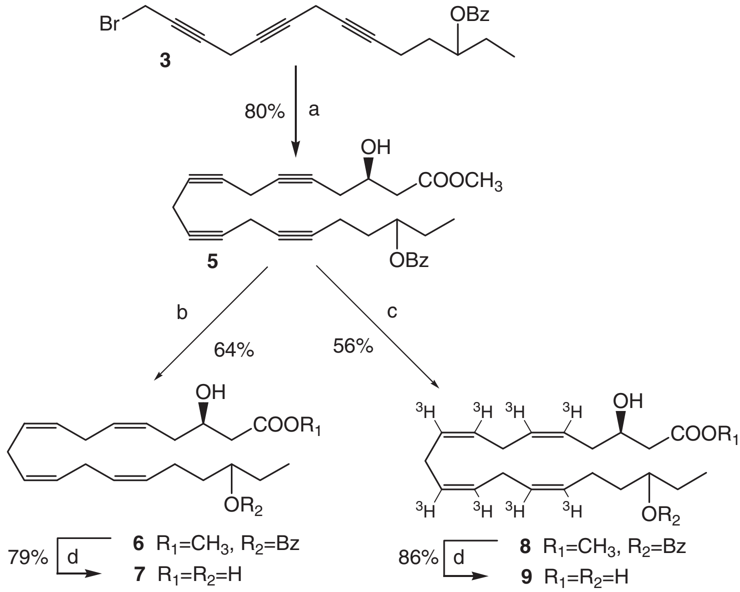
Acetylenic functions are widely used to build up tetraenoic systems selectively labelled with tritium on all double bond positions. Recently, we have reported a fast and efficient method for the preparation of 3(R)-HETE via its tetraacetylenic precursor.[6] Here we extend its application to the synthesis of tritium labelled 3(R)-HETE (**2**). As illustrated in Scheme 1, stereospecific



Scheme 1. (a) T_2/Lindlar's catalyst, quinoline, benzene, 400 hPa and (b) LiOH, MeOH, rt

tritiation (hydrogen–tritium mixture, 1% of tritium) of the triple bonds of methyl (*R*)-3-hydroxyeicosa-5,8,11,14-tetraynoate[6] (**1**) using Lindlar's catalyst and quinoline (catalyst/substrate ratio, 1.4:1) in benzene afforded [$^3H_8$]-**2** with an overall yield of 64% in two steps and a specific radioactivity of 1.65–1.75 Ci/mmol.

As illustrated in Scheme 2, [$^3H_8$]-3(*R*),18(*R*/*S*)-DiHETE (**9**) can be obtained in a similar manner starting from tetraacetylene **5**. Copper(I) mediated cross-coupling[7] of methyl 3(*R*)-hydroxy-5-hexynoate[6] (**4**) and rac-3-(benzoyloxy)-14-bromotetradeca-6,9,12-triyne[8] (**3**) resulted in methyl (3R,18R/S)-3-hydroxy-18-(benzoyloxy)eicosa-5,8,11,14-tetraynoate (**5**) in 80% yield. In order to optimize the conditions for preparation of [$^3H_8$]-(**9**) we first synthesised the unlabelled analogue **7**. Stereospecific hydrogenation of the triple bonds of **5** using Lindlar's catalyst and quinoline (catalyst/substrate ratio, 1.2:1) in benzene afforded, after reverse-phase HPLC methyl (3*R*,18*R*/*S*,5*Z*,8*Z*,11*Z*,14*Z*)-3-hydroxy-18-(benzoyloxy)eicosa-5,8,11,14-tetraenoate (**6**) in 64% yield. Interestingly, the increase in the amount of catalyst (catalyst/substrate ratio, 2:1) led to a significant decrease in the reaction time without any increase in the formation of over hydrogenated products. Simultaneous deprotection of both, carboxy and hydroxy groups of **6**, using NaOH in MeOH-H_2O solution proceeded well to give **7** in 56% yield. Tritiation of **5** was



Scheme 2. (a) 4, CuI, NaI, K_2CO_3, DMF, rt; (b) H_2/Lindlar's catalyst, quinoline, benzene, 440 hPa; (c) T_2/Lindlar's catalyst, quinoline, benzene and (d) NaOH, MeOH, rt

performed in benzene with a method used for the synthesis **2**. Subsequent deprotection yielded 36.5 mCi of **9** with a specific radioactivity of 1.70–1.80 Ci/mmol and a radiochemical purity of more than 97%.

In conclusion, we describe herein an effective method for the synthesis of [$^3H_8$]-3(*R*)-HETE and [$^3H_8$]-3(*R*),18(*R*/*S*)-DiHETE through a common synthetic route, which can also be applied to the preparation of analogues with high specific activity.

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